Edible Apple Film Wraps Containing Plant Antimicrobials Inactivate Foodborne Pathogens on Meat and Poultry Products

SADHANA RAVISHANKAR, LIBIN ZHU, CARL W. OLSEN, TARA H. MCHUGH, AND MENDEL FRIEDMAN

ABSTRACT: Apple-based edible films containing plant antimicrobials were evaluated for their activity against pathogenic bacteria on meat and poultry products. *Salmonella enterica* or *E. coli* O157:H7 (10^7 CFU/g) cultures were surface inoculated on chicken breasts and *Listeria monocytogenes* (10^6 CFU/g) on ham. The inoculated products were then wrapped with edible films containing 3 concentrations (0.5%, 1.5%, and 3%) of cinnamaldehyde or carvacrol. Following incubation at either 23 or 4 °C for 72 h, samples were stomached in buffered peptone water, diluted, and plated for enumeration of survivors. The antimicrobial films exhibited concentration-dependent activities against the pathogens tested. At 23 °C on chicken breasts, films with 3% antimicrobials showed the highest reductions (4.3 to 6.8 log CFU/g) of both *S. enterica* and *E. coli* O157:H7. Films with 1.5% and 0.5% antimicrobials showed 2.4 to 4.3 and 1.6 to 2.8 log reductions, respectively. At 4 °C, carvacrol exhibited greater activity than did cinnamaldehyde. Films with 3%, 1.5%, and 0.5% carvacrol reduced the bacterial populations by about 3, 1.6 to 3, and 0.8 to 1 logs, respectively. Films with 3% and 1.5% cinnamaldehyde induced 1.2 to 2.8 and 1.2 to 1.3 log reductions, respectively. For *L. monocytogenes* on ham, carvacrol films induced greater reductions than did cinnamaldehyde films at all concentrations tested. In general, the reduction of *L. monocytogenes* on ham at 23 °C was greater than at 4 °C. Added antimicrobials had minor effects on physical properties of the films. The results suggest that the food industry and consumers could use these films as wrappings to control surface contamination by foodborne pathogenic microorganisms.

Keywords: antimicrobial edible apple films, carvacrol, cinnamaldehyde, *E. coli* O157:H7, ham, *Listeria monocytogenes*, poultry, *Salmonella enterica*

Introduction

Contamination of processed foods, including poultry (Miranda and others 2008), by foodborne pathogens such as *Salmonella enterica*, *E. coli* O157:H7, and *Listeria monocytogenes* is an important safety issue for food processors and consumers. To meet this challenge, food-compatible, plant-derived antimicrobials could reduce surface contamination on meat and other foods.

S. enterica is an important foodborne pathogen responsible for gastrointestinal illness worldwide (Andrews and Bäumler 2005). Humans infected with *S. enterica* are reported to suffer from gastrointestinal symptoms such as acute diarrhea, vomiting, nausea, and stomach cramps. Poultry, egg, and peanut products have been implicated in foodborne salmonellosis (Andrews and Bäumler 2005; Centers for Disease Control and Prevention 2009). *E. coli* O157:H7 is a foodborne pathogen of concern in immunocompromised individuals, especially the elderly and young children (Smith and Fratamico 2005). The bacterium can cause kidney failure along with gastrointestinal symptoms such as diarrhea, nausea, vomiting, and abdominal pain. *E. coli* O157:H7 transmitted through a variety of food products, including hamburgers, apple cider, leafy greens, and poultry products, has caused numerous foodborne illness outbreaks (Smith and Fratamico 2005). According to the Center for Science in the Public Interest (CSPI) outbreak online database (http://www.cspinet.org/foodsafety/outbreak/pathogen.php), *E. coli* O157:H7 in contaminated chicken sickened 36 people in Arkansas in 2000, and in 1994 baked chicken was involved in an *E. coli* O157:H7 outbreak with 82 cases in Florida. *L. monocytogenes* is a foodborne pathogen of concern to immunocompromised individuals, especially pregnant women (Painter and Slutsker 2007). The organism can cause mild flu-like illness and can pass the blood-brain barrier causing meningitis and encephalitis. It can also adversely affect the fetus, causing abortions and stillbirths. Surface contamination of ready-to-eat meat and poultry products with *L. monocytogenes* is therefore an important food safety issue (Paoli and others 2005; Ryser and Marth 2007).

The incorporation of antimicrobials into edible films could serve as barrier for surface-contaminating microorganisms (Joerger 2007). To place our findings in perspective, we will briefly summarize the following relevant previous studies with films. Nisin-containing polyvinyl, polyethylene, and nylon films inactivated *S.* Typhimurium on fresh broiler skin (Natrajan and Sheldon 2000). Other studies describe attempts to control *L. monocytogenes* on ham by antimicrobial coatings (Jofre and others 2007; Marcos and others 2008). Films made of partially hydrolyzed sago starch and alginate mixture containing lemongrass oil inhibited *E. coli* O157:H7 (Maizura and others 2007). Cinnamon, clove, and lemongrass oils at 0.7%, or their active compounds, cinnamaldehyde, eugenol, and citral at 0.5% incorporated into alginate films reduced *E. coli* O157:H7 population by >4 logs on fresh cut Fuji apples (Raybaudi-Massilia and others 2008). Garlic oil incorporated into

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chitosan films showed antimicrobial activity against *E. coli*, *S. aureus*, *S. typhimurium*, *L. monocytogenes*, and *B. cereus* (Pranoto and others 2005a, 2005b). Garlic oil in alginate films at >0.2% concentrations exhibited antibacterial activity against *S. aureus* and *B. cereus* (Pranoto and others 2005b). Oregano oil at 2% in whey protein films was effective against *E. coli* O157:H7, *S. aureus*, *S. enteritidis*, *L. monocytogenes*, and *Lactobacillus plantarum* (Seydim and Sarikus 2006). Films with a 90/10 blend ratio of chitosan and polyethylene oxide exhibited strong antimicrobial properties (Zivanovic and others 2007).

Our previous studies revealed that edible apple- and tomato-based films, prepared from apple and tomato slurries (purees) and containing low levels of plant essential oils (oregano, cinnamon, lemongrass) or their major constituents (carvacrol, citral, and cinnamaldehyde) induced reductions in *E. coli* O157:H7 (Rojas-Graü and others 2006, 2007; Du and others 2008a, 2008b). These studies facilitated optimizing effective levels of the plant antimicrobials in fruit and vegetable films against foodborne pathogens.

Depending on geographical source, the carvacrol content of oregano oil ranges up to 85% and cinnamaldehyde content of cinnamon oil up to 86% (Friedman and others 2004, Parthasarathy and Chempakam 2008; Teuscher 2006).

To determine whether edible antimicrobial fruit and vegetable films will inactivate pathogens on food surfaces, the objective of the present study, therefore, was to determine the antimicrobial efficacy of carvacrol, the main ingredient of oregano oil, (used to flavor salad dressings, tomato sauces, pizzas, and so on) (McGee 2004; Parthasarathy and Chempakam 2008; Teuscher 2006) and cinnamaldehyde, the main ingredient of cinnamon oil (used as a flavoring agent in numerous foods) (Friedman and others 2000) in apple-based edible films against *S. enterica* and *E. coli* O157:H7 on chicken breast surfaces as well as against *L. monocytogenes* on ham surfaces. To our knowledge, no published studies have as yet addressed the inactivation of pathogens by apple-based antimicrobial films on surfaces of meat and poultry products.

Materials and Methods

Bacterial cultures and media

Because we previously reported that different strains of *E. coli* O157:H7 and *S. enterica* showed similar susceptibilities to the plant antimicrobials in phosphate buffer (Friedman and others 2002, 2004), we exposed the films to only a single strain of each pathogen. Test organisms used include *S. enterica* serovar *Enteritidis*, *E. coli* O157:H7 (ATCC strain 35150), and *L. monocytogenes* (strain 101M; serotype 4b; beef and pork sausage isolate). Stock cultures of each organism were maintained in cryovials (Microbank^{TM} Austin, Tex., U.S.A.) at –80 °C and activated by transferring 100 μL into tryptic soy broth with 0.6% yeast extract (TSBYE; Difco Laboratories, Sparks, Md., U.S.A.). The bacterial cultures were maintained in TSBYE at 4 °C with biweekly transfers. For experimental use, an overnight culture of each test organism was grown in TSBYE at 37 °C for 18 to 24 h with shaking. All dilutions were made in buffered peptone water (BPW; Difco Laboratories). Enumerations for *S. enterica* were done by plating on xylose lysine desoxycholate agar (XLD; Difco Laboratories). Enumerations for *E. coli* O157:H7 were done by plating on sorbitol MacConkey agar (SMAC; Difco Laboratories). *L. monocytogenes* was enumerated by plating on tryptic soy agar (TSA; Difco Laboratories).

Food products and antimicrobials

The test products used in our study were skinless, boneless chicken breast, and cooked ham obtained from local stores in Tucson, Ariz., U.S.A. These were kept frozen and thawed before use. The tested antimicrobials included carvacrol and cinnamaldehyde at 0.5%, 1.5%, and 3% concentrations incorporated into the apple films. The films were made at the USDA-ARS-WRRC facility in Albany, Calif., U.S.A., as described subsequently.

Edible apple film preparation

A 3% solution of high methoxyl pectin 11400 (TIC Gums, Belcamp, Md., U.S.A.) was prepared by mixing 30 g of pectin into 970 g of water. Mixing was done at low speed for 45 min in a Kitchen Aid mixer. The solution was then placed in a 90 °C water bath for 15 min at low speed on a Kitchen Aid mixer until the pectin was solubilized. The 3% pectin solution was refrigerated until needed.

The apple solution was prepared using the Kitchen Aid mixer by adding golden delicious apple puree (Sabroso Co, Medford, Oreg., U.S.A.) and vegetable glycerin (Starwest Botanicals, Rancho Cordova, Calif., U.S.A.) to the pectin solution and mixing at low speed for 15 min. The apple puree used to prepare the films contained 12% glycerin.

The apple solution was divided into 4 parts. Carvacrol (>98%, Sigma, St. Louis, Mo., U.S.A.) or cinnamaldehyde (93%, Sigma) were added to 3 portions at 0.5%, 1.5%, and 3% (w/w). The 4th portion was used as a control without antimicrobials. The solutions were homogenized on the Kinematica Polytron (Beckman Instruments Inc., Westbury, N.Y., U.S.A.) for 2.5 min between 16000 and 20000 rpm. The solutions was then degassed and cooled.

The films were prepared by placing polyester film (PET) on a glass plate (30.5 × 30.5 cm). This was followed by placing the apple solution (58 to 61 g) on the PET film. The film was then cast using a draw down bar. The wet film was 20.3 cm wide and 1.1 mm thick. The films were then dried for approximately 14 h at room temperature (23 to 25 °C). Samples for antimicrobial studies were prepared by placing a watch glass (50 mm in diameter) over the film and tracing over the film with a single edge razor. The films are water soluble (results not shown).

Effect of edible apple film wraps containing antimicrobials on *S. enterica* and *E. coli* O157:H7 on chicken breast, and on *L. monocytogenes* on ham

Chicken breast samples were cut into 10 g pieces (3 × 3 cm) and quickly dipped into boiling water (100 °C) for 40 s to inactivate the background flora. These chicken samples were cooled and kept in petri dishes with lids open under a bio-hood, and dried for 30 min. The samples were then surface-inoculated with a diluted overnight culture (0.1 mL; 10^7 CFU/mL) of either *S. enterica* or *E. coli* O157:H7. The inoculum was dispersed on the surface as droplets with a sterile pipette. The inoculated samples were then dried under a bio-hood for 30 min. They were then surface-wrapped with edible apple films containing 3 concentrations of carvacrol or cinnamaldehyde (Figure 1).

The sliced ham samples were cut into 5 g pieces and transferred to sterile petri dishes. The samples in petri dishes with lids open were kept under a bio-hood and dried for 20 min. Because cooked ham obtained from the grocery stores had very low background levels (<100 CFU/g), they were not dipped in hot water like the chicken samples. The ham samples in petri dishes were inoculated with a diluted overnight culture (0.1 mL; 10^6 CFU/mL) of *L. monocytogenes* on one side. The inoculum was dispersed on the surface as droplets with a sterile pipette. The inoculated samples were then dried under the bio-hood for 30 min. They were then flipped over and the other side, inoculated in a similar way, dried under the bio-hood for 30 min, and then surface-wrapped with one of the edible apple films.

The wraps touched and covered all surfaces of the chicken breasts and ham. The inoculated, wrapped samples were stored at either room (23 °C) or refrigeration (4 °C) temperatures for 72 h. After 72 h, samples were taken for plating and enumeration of survivors. Edible apple film wraps were carefully removed from the chicken and ham samples using sterile forceps. Chicken (10 g) and ham (5 g) samples were stomached in BPW (90 and 95 mL, respectively) in WhirlPak bags at normal speed for 1 min. Serial dilutions in BPW were plated on appropriate media (XLD, SMAC, or TSA), as described earlier. The plates were incubated at 37 °C for 24 to 48 h and enumerated. Control chicken and ham samples wrapped with apple films without antimicrobials were included in all experiments.

Effect of edible apple film wraps containing antimicrobials on normal microflora on noninoculated raw chicken breast

For this study, chicken breasts bought from the grocery stores were used without being dipped in hot water. Noninoculated raw chicken breast samples (10 g) were wrapped with edible apple films containing 1.5% or 3% carvacrol or cinnamaldehyde and stored at 4 °C for 72 h. These samples were stomached in 90 mL BPW at normal speed for 1 min, serially diluted as needed in BPW, and plated on TSA, XLD, and SMAC. Plates were incubated at 37 °C for 24 to 48 h and checked for colony forming units (CFU). Samples wrapped with edible apple films containing no antimicrobials served as controls. Unwrapped raw chicken samples were also plated.

Physical properties of edible films

Physical properties of the films used in the present study were determined by standard ASTM methods as described in detail elsewhere (Du and others 2008a, 2008b).

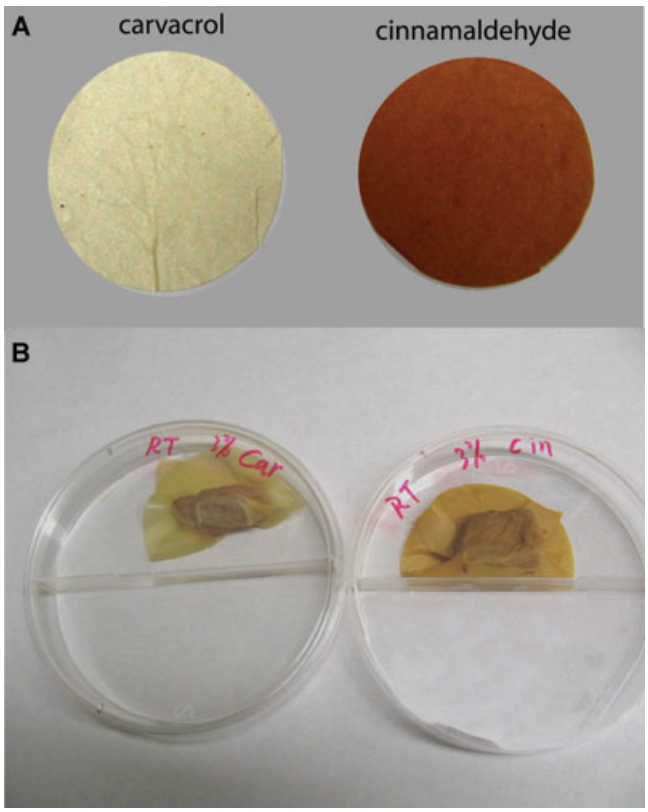

Figure 1—Photographs of apple films with added carvacrol or cinnamaldehyde. (A) Films after production. (B) Poultry breast wrapped with films.

Statistical analysis

Three or more replicates were carried out for each experiment. For microbial data, mean and standard deviation values were calculated for the surviving bacterial populations from various samplings. For edible film physical properties and microbial counts, data were analyzed by one-way analysis of variance (ANOVA) using Minitab version 13.31 software (Minitab Inc., State College, Pa., U.S.A.). Tukey's test was used to determine differences at the 5% significance level (SAS 1999).

Results and Discussion

The use of edible antimicrobial films on meat, poultry, and seafood can offer the following advantages to the consumer: prevention of moisture loss; control of dripping of juices, thereby avoiding cross contamination; reduction of lipid oxidation (rancidity) and browning (discoloration); reduction in microbial load; and prevention of losses of volatile flavors and foreign odor pickup (Gennadios and others 1997).

As mentioned in the Introduction, many studies have investigated antimicrobial activities of films containing plant compounds. However, most of these were done *in vitro*. In the present study, we investigated antimicrobial activities of cinnamaldehyde- and carvacrol-containing edible apple films against *E. coli* O157:H7 and *S. enterica* on chicken breast, and *L. monocytogenes* on ham.

Effect of edible apple-film wraps containing antimicrobials on *S. enterica* and *E. coli* O157:H7 on chicken breast

It should be noted that sterility cannot be achieved by dipping in hot water. However, since the chicken samples in our study had low background counts (up to 10^{3-4} CFU/g), even the 40 s dipping used, inactivated the background flora. It is possible that the background flora in our samples was heat sensitive. There were no survivors of background microflora or pathogens on chicken samples heated for 40 s at 100 °C and plated on TSA, XLD, and SMAC. The detection limit of the microbiological assays was 1 log CFU/g. Control films without antimicrobials did not inactivate the pathogens. Compared to control films, the antimicrobial-containing films showed a strong concentration-dependent inhibition of both *E. coli* O157:H7 and *S. enterica* on chicken breast samples after 72 h storage at both room and refrigeration temperatures (Table 1). In some cases, the bacteria multiplied at 23 °C. In all cases, growth was higher on chicken samples wrapped with control films without antimicrobials. At 23 °C, films with 3% carvacrol induced the highest reduction of *E. coli* O157:H7 (6.8 logs) and *S. enterica* (4.6 logs). At 4 °C, 3% carvacrol induced an approximately 3 log reduction, with no survivors detected for both organisms.

At 23 and 4 °C, films containing 3% cinnamaldehyde induced 4.3 and 2.8 log reductions of *S. enterica*, respectively. The corresponding reductions in case of *E. coli* O157:H7 were 5.2 and 1.2 logs.

With 1.5% carvacrol, the reductions in *S. enterica* populations at 23 and 4 °C were 2.4 and 1.6 logs, respectively. The corresponding reductions in *E. coli* O157:H7 were 4.3 and 3 logs, respectively. With 1.5% cinnamaldehyde, the reductions for both organisms were higher at 23 °C (>3 logs) than at 4 °C (>1 log).

At 23 °C, films containing 0.5% carvacrol induced greater reductions of *E. coli* O157:H7 (2.8 logs) than of *S. enterica* (1.7 logs). At 4 °C, 0.5% carvacrol induced about 1 log reduction of *E. coli* O157:H7 and 0.8 log reduction of *S. enterica*. At 23 °C, 0.5% cinnamaldehyde induced a 1.6 log reduction of *S. enterica* and a 1.8

log reduction of *E. coli* O157:H7. However, limited or no reductions occurred with both organisms at 4 °C.

Effect of edible apple film wraps containing antimicrobials on normal microflora on noninoculated raw chicken breast

It was also of interest to find out whether the antimicrobial films would inactivate the natural microflora on raw chicken consisting of spoilage (*Lactobacilli, Pseudomonas* spp.) and other unknown bacteria (Del Rio and others 2004; Dominguez and Schaffner 2007; No and others 2007; Ponce and others 2008). The raw chicken samples without films contained 6.3×10^1 CFU/g organisms at 0 h. This population grew to 7.6×10^4 CFU/g during storage at 4 °C for 72 h. Films without the antimicrobials had 3×10^1 CFU/g organisms at 0 h. Population growth also occurred in these samples to 2.8×10^3 CFU/g by 72 h of storage. However, no surviving microorganisms (<1 log CFU/g) were detected on chicken samples wrapped with antimicrobial films (both 1.5% and 3% carvacrol and cinnamaldehyde) at 0 and 72 h.

No attempt was made to identify the organisms with regard to the background flora grown on media plates. However, it was evident from these experiments that the antimicrobials incorporated into the edible films were also effective against the natural microflora found on raw chicken.

Effect of edible apple film wraps containing antimicrobials on *L. monocytoge*nes on ham

Low levels of background microflora were present on cooked ham (<100 CFU/g). These low levels on ham (due to their colony morphology being different from *Listeria*) did not interfere with enumeration of *L. monocytogenes*. We also confirmed suspect colonies by plating them on modified Oxford formulation (MOX; a selective medium for *Listeria*) and using rapid tests for *Listeria*.

Table 2 shows that the antimicrobial edible apple films against *L. monocytogenes* on the surface of ham were more effective at 23 °C than at 4 °C. The table also shows that at 4 °C, carvacrol-containing films exhibited stronger activity than did films containing cinnamaldehyde. At 23 °C, the reductions in the surviving *L.*

Table 1 — Survival of *S. enterica* serovar *Enteritidis* and *E. coli* O157:H7 (log CFU/g) on chicken breast samples wrapped with edible apple films containing carvacrol (CAR) and cinnamaldehyde (CIN) at various concentrations and stored for 72 h at 23 and 4 °C.

		Initial count (mean ± SD)	Treated, 72 h (mean ± SD)	Control, 72 h (mean ± SD)	Log CFU/g reduction
Salmonella Enteritidis					
23 °C	3% CAR	2.8 ± 0.5	4.1 ± 0.1[a]	8.7 ± 0.5	4.6
	1.5% CAR	4.3 ± 0.3	6.1 ± 0.3	8.5 ± 0.5	2.4
	0.5% CAR	4.5 ± 0.2	6.8 ± 0.2	8.5 ± 0.5	1.7
	3% CIN	4.2 ± 0.3	3.8 ± 0.6	8.1 ± 0.9	4.3
	1.5% CIN	4.5 ± 0.0	6.1 ± 0.7	9.2 ± 0.9	3.1
	0.5% CIN	4.7 ± 0.1	7.3 ± 0.7	8.9 ± 1.1	1.6
4 °C	3% CAR	2.8 ± 0.5	< 1.0 ± 0.0	4.3 ± 0.0	3.3
	1.5% CAR	4.3 ± 0.3	2.7 ± 0.4	4.3 ± 0.3	1.6
	0.5% CAR	4.5 ± 0.2	3.5 ± 0.3	4.3 ± 0.1	0.8
	3% CIN	4.2 ± 0.3	1.4 ± 0.7	4.2 ± 0.2	2.8
	1.5% CIN	4.5 ± 0.0	2.7 ± 0.5	4.0 ± 0.5	1.3
	0.5% CIN	4.7 ± 0.1	4.1 ± 0.2[NS]	4.0 ± 0.5	−0.1
E. coli O157:H7					
23 °C	3% CAR	3.0 ± 0.5	< 1.0 ± 0.0	7.8 ± 0.6	6.8
	1.5% CAR	4.5 ± 0.0	4.1 ± 0.6	8.4 ± 0.8	4.3
	0.5% CAR	4.6 ± 0.1	5.5 ± 0.8	8.3 ± 1.0	2.8
	3% CIN	4.5 ± 0.2	2.3 ± 0.2	7.5 ± 0.7	5.2
	1.5% CIN	4.7 ± 0.1	4.0 ± 0.3	7.8 ± 0.5	3.8
	0.5% CIN	4.8 ± 0.1	6.6 ± 1.1	8.4 ± 1.2	1.8
4 °C	3% CAR	3.0 ± 0.5	< 1.0 ± 0.0	4.0 ± 0.1	3.0
	1.5% CAR	4.5 ± 0.0	< 1.0 ± 0.0	4.0 ± 0.2	3.0
	0.5% CAR	4.6 ± 0.1	3.3 ± 0.6	4.3 ± 0.4	1.0
	3% CIN	4.5 ± 0.2	3.0 ± 0.2	4.2 ± 0.4	1.2
	1.5% CIN	4.7 ± 0.1	3.3 ± 0.5	4.5 ± 0.5	1.2
	0.5% CIN	4.8 ± 0.1	4.3 ± 0.7[NS]	4.5 ± 0.5	0.2

[a]Data reported are mean values ± standard deviations. All means are significantly different at $P < 0.05$ except those with the superscript NS (not significant).

Table 2 — Survival of *L. monocytogenes* 101M (log CFU/g) on ham wrapped with edible apple films containing carvacrol (CAR) and cinnamaldehyde (CIN) at various concentrations and stored for 72 h at 23 and 4 °C.

		Initial count (mean ± SD)	Treated, 72 h (mean ± SD)	Control, 72 h (mean ± SD)	Log reduction (CFU/g)
23 °C	3% CAR	3.5 ± 0.3	1.4 ± 0.2[a]	3.7 ± 0.2	2.3
	1.5% CAR	4.1 ± 0.0	1.8 ± 0.2	3.7 ± 0.2	1.9
	0.5% CAR	4.6 ± 0.2	3.2 ± 0.2[NS]	3.7 ± 0.2	0.5
	3% CIN	4.3 ± 0.1	2.2 ± 0.3	3.7 ± 0.2	1.5
	1.5% CIN	4.3 ± 0.2	3.8 ± 0.2[NS]	3.7 ± 0.2	−0.1
	0.5% CIN	4.7 ± 0.2	4.0 ± 0.03[NS]	3.7 ± 0.2	−0.3
4 °C	3% CAR	3.5 ± 0.3	2.9 ± 0.2	5.1 ± 0.02	2.2
	1.5% CAR	4.1 ± 0.0	3.8 ± 0.02	5.1 ± 0.02	1.3
	0.5% CAR	4.6 ± 0.2	4.7 ± 0.02[NS]	5.1 ± 0.02	0.4
	3% CIN	4.3 ± 0.1	4.9 ± 0.1	5.1 ± 0.02	0.2
	1.5% CIN	4.3 ± 0.2	5.0 ± 0.1[NS]	5.1 ± 0.02	0.1
	0.5% CIN	4.7 ± 0.2	5.1 ± 0.03[NS]	5.1 ± 0.02	0.0

[a]Data reported are mean values ± standard deviations. All means are significantly different at $P < 0.05$ except those with the superscript NS (not significant).

Table 3 — Water vapor permeability of edible apple films containing either carvacrol or cinnamaldehyde at concentrations of 0%, 0.5%, 1.5%, or 3% at temperatures between 24 and 26 °C and 0% relative humidity.

Apple films	Concentration (% w/w)	Thickness (mm)	Relative humidity (%)	Permeability (g-mm/kPa-h-m^2)
Control	0.0	0.131 ±0.01^{a,A}	77.9 ±1.1^{a,A}	3.91 ±0.28^{a,A}
Carvacrol	0.5	0.126 ±0.01^{a}	77.5 ±1.5^{a}	3.87 ±0.08^{a}
Carvacrol	1.5	0.127 ±0.005^{a}	77.2 ±0.8^{a}	3.99 ±0.15^{a}
Carvacrol	3.0	0.130 ±0.004^{a}	78.0 ±1.1^{a}	3.88 ±0.24^{a}
Cinnamaldehyde	0.5	0.126 ±0.007^{A}	78.3 ±1.7^{A}	3.70 ±0.23^{A,B}
Cinnamaldehyde	1.5	0.125 ±0.006^{A}	79.0 ±1.8^{A}	3.51 ±0.27^{B}
Cinnamaldehyde	3.0	0.134 ±0.004^{A}	78.7 ±0.9^{A}	3.85 ±0.15^{A,B}

^{a}Represents no significant differences between the control and films with carvacrol, $N = 7$, $P < 0.05$ using Tukey's mean comparison.
^{A,B}Represents significant differences between the control and films with cinnamaldehyde, $N = 7$, $P < 0.05$ using Tukey's mean comparison.

Table 4 — Color changes of edible apple films containing either carvacrol or cinnamaldehyde at concentrations of 0%, 0.5%, 1.5%, or 3% at temperatures between 24 and 26 °C and 0% relative humidity.

Apple films	Concentration (% w/w)	L^*	a^*	b^*	Whitish index
Control	0.0	86.8 ±0.4^{a,A}	−0.90 ±0.15^{a,A}	20.7 ±1.8^{b,C}	75.4 ±1.7^{a,A}
Carvacrol	0.5	86.0 ±0.3^{b}	−0.95 ±0.16^{a}	20.8 ±1.8^{b}	74.9 ±1.5^{a}
Carvacrol	1.5	85.9 ±0.4^{b,c}	−1.32 ±0.14^{b}	23.6 ±0.9^{a}	72.5 ±0.8^{b}
Carvacrol	3.0	85.5 ±0.6^{c}	−1.14 ±0.19^{b}	25.0 ±1.3^{a}	71.1 ±1.3^{b}
Cinnamaldehyde	0.5	85.8 ±0.6^{B}	−1.44 ±0.29^{B}	25.8 ±2.3^{B}	70.5 ±2.3^{B}
Cinnamaldehyde	1.5	85.4 ±0.3^{B,C}	−2.05 ±0.11^{C}	32.7 ±0.5^{A}	64.2 ±0.5^{C}
Cinnamaldehyde	3.0	85.0 ±0.7^{C}	−1.20 ±0.59^{A,B}	33.3 ±0.9^{A}	63.4 ±1.0^{C}

^{a,b,c}Represents significant differences between the control and films with carvacrol, $N = 10$, $P < 0.05$ using Tukey's mean comparison.
^{A,B,C}Represents significant differences ($P < 0.05$) between the control and films with cinnamaldehyde, $N = 10$, $P < 0.05$ using Tukey's mean comparison.

Table 5 — Mechanical properties of edible apple films containing either carvacrol or cinnamaldehyde at concentrations of 0%, 0.5%, 1.5%, or 3% at temperatures between 24 and 26 °C and 0% relative humidity.

Apple films	Concentration (% w/w)	Tensile strength (MPa)	Modulus (MPa)	Elongation (%)
Control	0.0	3.44 ±0.27^{a,B}	5.10 ±0.43^{a,A,B}	47.01 ±3.77^{a,B}
Carvacrol	0.5	3.51 ±0.32^{a}	4.87 ±0.68^{a}	47.94 ±3.56^{a}
Carvacrol	1.5	3.13 ±0.20^{b}	4.76 ±0.69^{a}	47.47 ±2.82^{a}
Carvacrol	3.0	2.96 ±0.21^{b}	4.15 ±0.46^{b}	48.86 ±2.04^{a}
Cinnamaldehyde	0.5	3.69 ±0.26^{A}	5.28 ±0.50^{A}	48.88 ±3.09^{A,B}
Cinnamaldehyde	1.5	3.51 ±0.22^{B}	5.05 ±0.68^{A,B}	49.10 ±2.83^{A,B}
Cinnamaldehyde	3.0	3.19 ±0.15^{C}	4.72 ±0.35^{B}	49.88 ±1.83^{A}

^{a,b}Represents significant differences between the control and films with carvacrol, $N = 15$, $P < 0.05$ using Tukey's paired comparison.
^{A,B,C}Represents significant differences between the control and films with cinnamaldehyde, $N = 15$, $P < 0.05$ using Tukey's paired comparison.

monocytogenes population with 3%, 1.5%, and 0.5% carvacrol films were 2.3, 1.9 and 0.5 logs, respectively. However, with films containing cinnamaldehyde, only a 1.5 log reduction was seen with 3% and no reduction with 1.5% or 0.5% concentrations.

At 4 °C, films containing carvacrol showed about 2, 1, and 0.5 log reductions in *L. monocytogenes* populations at 3%, 1.5%, and 0.5% concentrations, respectively. Very limited reduction was observed with cinnamaldehyde. These results indicate that, just as is the case with chicken breast described earlier, carvacrol also exhibited higher activity than did cinnamaldehyde against *L. monocytogenes* on cooked ham. The increased resistance of *L. monocytogenes* at 4 °C towards the antimicrobial films could possibly be attributed to the psychrotrophic nature of the organism.

Physical properties of edible apple films

To facilitate applications, we also evaluated the physical properties of the edible apple films. Table 3 to 5 summarize experimental data on physical properties of films used in the present study evaluated by methods described elsewhere (Du and others 2008a, 2008b). Addition of either carvacrol or cinnamaldehyde to the purees used to make the films had minor effects on thickness, tensile strength, modulus, elongation, and humidity.

The data also show that there was a significant difference in permeability between carvacrol and cinnamaldehyde. As the concentration of cinnamaldehyde increased, the water vapor permeability also increased. A small significant difference was also apparent in the water vapor permeability of films containing different amounts of cinnamaldehyde. Cinnamaldehyde films had better barrier properties (water vapor release or absorption) than did carvacrol films.

The following parameters describe color changes in the films: L^* value, a measure of lightness, a^* value of greenness, and b^* value of yellowness (Rojas-Graü and others 2006, 2007; Du and others 2009). The L^*, a^*, and b^* values of apple films formulated in the present study with the 2 antimicrobials indicate a change from the control to a light yellowish–greenish color. Cinnamaldehyde reduced the Whitish Index to a greater extent than did carvacrol.

Conclusions

The results of the present study show that, in general, carvacrol exhibited stronger activity against both organisms on poultry meat surfaces at 4 °C than did cinnamaldehyde. At 23 °C, the reductions of *S. enteritidis* were similar for both carvacrol and cinnamaldehyde. However, higher reductions of *E. coli* O157:H7 were seen with carvacrol at 23 °C compared to cinnamaldehyde. Carvacrol also exhibited stronger activity against *L. monocytogenes* on ham than did cinnamaldehyde.

It is also relevant to note that in previous studies we found that carvacrol also inactivated antibiotic-resistant *Campylobacter jejuni* in a phosphate buffer (Ravishankar and others 2008) and facilitated inactivation/inhibition of *E. coli* O157:H7 and of potentially carcinogenic heterocyclic amines in grilled ground beef patties

(Friedman and others 2009). This study demonstrates the potential of edible apple films containing the 2 plant-derived antimicrobials to inactivate pathogenic bacteria on contaminated chicken breast, cooked ham, and possibly other contaminated food products. To demonstrate the efficacy of the antimicrobial edible apple films, the wrapped foods were evaluated at 2 temperatures. The findings complement and extend related observations on the inactivation of foodborne pathogens by the same antimicrobials added to ground meats (Juneja and Friedman 2007, 2008) and provide a scientific rationale for large-scale application of apple-based antimicrobial films to improve microbial food safety. Sensory properties of poultry and ham wrapped with edible apple films merit further study.

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